An Overview on Emergence of Virulent *Vibrio parahaemolyticus* - A Global Public Health Concern

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(Received 21.03.2014; accepted 10.10.2014)

With the increasing habit of seafood intake, there is an upsurge in gastroenteritis and food poisoning cases due to toxic and microbial agents harboured in seafood. In this regard, vibrios of seafood origin, especially *Vibrio parahaemolyticus* is one of the leading etiologic agents of foodborne gastroenteritis and has attained paramount importance globally (Fujino *et al.*, 1953; Sakazaki *et al.*, 1971; Martinez-Urtaza *et al.*, 2013). Its occurrence in clinical cases (Pal *et al.*, 1984; Bag *et al.*, 1999, Chowdhury *et al.*, 2000a; 2000b) as well as from saline water fishes (Anjay *et al.*, 2011; 2013) has also been reported in India.

Moreover, since 1995, with the occurrence and identification of O3:K6 serotype, this organism has gained increased attention as an emerging public health zoonotic pathogen in global context including India (Chowdhury *et al.*, 2000a). The unique combination of pathogenicity and halophilism manifested by *V. parahaemolyticus* has generated much interest because of the food safety and public health issues. This review paper attempts to note the magnitude of occurrence of pathogenic population of *V. parahaemolyticus* and its pandemic clone in food chain and aquatic bodies as well as to highlight this emerging foodborne zoonotic pathogen by identifying the cardinal virulence determinant of this organism.

**Ecological distribution**

Environmental factors *viz.*, water temperature, salinity, zooplankton blooms, tidal flushing and dissolved oxygen play an important role in influencing the survival and proliferation of this pathogen and dictate its spatial and temporal distribution (Kaneko and Colwell, 1973). However, it is vulnerable to environmental stresses and rapidly gets inactivated at 48°C and 55°C with salinity below 0.5% or pH 4.0 (Beuchat, 1975). Water temperatures have been shown to influence the growth of *V. parahaemolyticus* (Kaneko and Colwell, 1973 and 1975; Thompson *et al.*, 1976; Kelly and Stroh, 1981). The importance of water temperature in the epidemiology of infections is reflected by the fact that most outbreaks occur during the warmer months. In tropical countries, in contrast, the seasonality of *V. parahaemolyticus* is less defined, with infection occurring throughout the year. Most *V. parahaemolyticus* outbreaks between 1973 and 1998 in the United States occurred during the warmer months and were attributed to seafood, particularly oysters and other shellfish, and the median attack rate among persons who consumed the implicated seafood was 56% (Morris *et al.*, 1981). It is postulated that warmer sea temperatures (the El Nino effect) has resulted in the emergence of more virulent serotypes (Daniels *et al.*, 2000).

**Identification of the organism**

*Vibrio parahaemolyticus*, is a curved, rod-shaped, Gram-negative halophilic bacterium that inhabits temperate and tropical estuarine, marine and coastal environments worldwide (DePaola *et al.*, 2000; Kaysner and DePaola, 2001; Nishibuchi and DePaola, 2005; Faruque and Nair, 2006). It is distributed in freshwater environs, albeit in low frequencies, than in seawater (Sarkar *et al.*, 1985; Charles-Hernandez *et al.*, 2006). It is oxidase positive, facultatively anaerobic, non-spore forming and motile with a single polar flagellum.

**Cultural isolation and biochemical characterization**

The cultural isolation of *V. parahaemolyticus* can be performed from both clinical and environmental samples where samples are required to be transported in the Cary–Blair medium or directly enriched in alkaline peptone water (APW) and incubated for 16-18 h at 35-37°C (Elliot *et al.*, 1992). All kinds of diluents and enrichment media should be supplemented with 3% NaCl. The growth of *V. parahaemolyticus* in glucose salt
teepol broth (GST) or APW at 37°C is markedly faster than that of marine and faecal flora (Dupray and Cormier, 1983).

Isolation of Vibrio species has been enhanced by the development of media, which are highly selective for vibrios. Kobayashi et al. (1963) developed thiosulfate citrate bile salts sucrose (TCBS) agar by the modification of the selective medium of Nakanishi (1963). It is the primary plating medium universally used for the selective isolation of vibrios such as Vibrio cholerae and V. parahaemolyticus from a variety of clinical and nonclinical specimens (Clesceri et al., 1998; Downes and Ito, 2001). On TCBS agar, V. parahaemolyticus produces non-sucrose fermenting typical round (2-3 mm in diameter), green or blue center colonies (Elliot et al., 1983). Ito, 2001). On TCBS agar, V. parahaemolyticus is reported to be mannitol positive and arginine negative, which laid its characterization biochemically by Kaper’s multi-test tube medium and Iida, 1993). This gene was shown to produce an acid (yellow) but with an alkaline (purple) slant (Kaper’s et al., 1980). Usually by cultural isolation, the incidence of pathogenic V. parahaemolyticus has been reported to be less than 1-2% among environmental samples (Kelly and Stroh, 1988; Honda and Iida, 1993).

**Molecular characterisation**

Comparative study of cultural and PCR methods for identification of V. parahaemolyticus from shrimp revealed that PCR would be very useful and superior to conventional method (Karunasagar et al., 1997). This technique has potential to detect even atypical strains showing variation in biochemical reactions.

**a) Species-specific PCR assay (VP-toxR)**

The toxR gene is a regulatory gene controlling the expression of the genes encoding important extracellular virulence factors and other virulence-associated genes in vibrios (Lin et al., 1993). This gene was shown to stimulate expression of the tdh gene encoding thermostable direct hemolysin; a major virulence factor of this microorganism (Lin et al., 1993). Reich and Schoolnik (1994) cloned and analyzed the toxR gene sequences from V. fisheri and two other species of Vibrio and stated that the toxR gene appeared to be well conserved among Vibrio species. The degree of homology of the toxR gene between V. parahaemolyticus and V. cholerae is 52% and is much lower than that of the rRNA gene (91 to 92% identity) (Kita-Tsukamoto et al., 1993; Lin et al., 1993). The toxR gene fragment (~368 bp), which is specific for V. parahaemolyticus presents in all strains and thus can be used as a species specific marker for all strains of V. parahaemolyticus (Kim et al., 1999; Dileep et al., 2003).

**b) Virulence factors**

**i) Thermostable direct haemolysin (tdh)**

Thermostable direct haemolysin (TDH) is commonly associated with clinical strains and has been considered an important virulence factor (Miwatani and Takeda, 1976). The isolates of V. parahaemolyticus, which produce thermostable direct haemolysin are referred as Kanagawa phenomenon positive (KP+) (Wagatsuma, 1974; Takeda, 1983) as it is reported to be responsible for the induction of β-hemolysis on Wagatsuma blood agar containing human erythrocyte (Wagatsuma, 1968). Miyamoto et al. (1969) stated that the ability to produce β-type haemolysis around colonies on Wagatsuma blood agar (a special blood medium) is used traditionally to determine the production of TDH by V. parahaemolyticus. However, the blood agar test is not easily determined and may give false negative results. Honda et al. (1989) reported an enzyme-linked immunosorbent assay (ELISA) for detection of TDH-producing strains of V. parahaemolyticus.

DNA probes and oligonucleotide probes specific for the gene encoding TDH have been described and used as hybridization probes (Nishibuchi et al., 1985; Nishibuchi et al., 1986; Yamamoto et al., 1992). However, the tdh probes also hybridized with tdh-like genes were also possessed by V. hollisae, V. mimicus and V. cholerae non-01.

TDH have also been reported to produce enterotoxin, cardiotoxic and cytotoxic activities along with hemolytic activity (Wagatsuma, 1974; Shirai et al., 1990; Nishibuchi et al., 1992; Honda and Iida, 1993). Five sequence variants of tdh (named tdh1 to tdh5) have been identified, however only tdh2 appears to have a high level of transcription (Nishibuchi and Kaper, 1990; Nakaguchi and Nishibuchi, 2005). Honda et al. (1992) reported that TDH damages the eukaryotic cells by acting as a pore forming toxin that alters the ion balance of cells. Nishibuchi et al. (1992) stated that the presence of thermostable direct hemolysin (TDH) is a proven virulence factor, which can cause gastroenteritis.

Gastroenteritis caused by V. parahaemolyticus results in strong systemic and mucosal B-cell responses to TDH and lipopolysaccharide; both antigens also induce an increase in the presence of immunoglobulin M antibody-secreting cells, which suggests that this is a primary response to the antigen (Qadri et al., 2003). In a recent study, it was shown that irrespective of TDH production, V. parahaemolyticus profoundly disturbs epithelial barrier function in Caco-2 cells due to the involvement of another virulence factor(s) (Lynch et al., 2005). The overall mechanism of pathogenesis by V. parahaemolyticus, however, remains unclear.
ii) TDH-related hemolysin (TRH):
More than 90% of clinical *V. parahaemolyticus* isolates possess *tdh* (DePaola *et al.*, 1990; Kaysner *et al.*, 1990; DePaola *et al.*, 2000; Wong *et al.*, 2000); however, some clinical stains of *V. parahaemolyticus* that are lacking *tdh* but having TDH-related hemolysin (TRH) virulence factor encoded by the *trh* gene was discovered by Honda *et al.* (1987) and Honda *et al.* (1988). As the *trh* gene sequence can vary from strain to strain, the *trh* gene sequences can be clustered into two subgroups, represented by *trh1* and *trh2* (Kishishita *et al.*, 1992). Okuda *et al.* (1997) reported that most of the strains isolated between 1982 and 1995 had either *trh1* or the *trh2* gene and produce urease. Iida *et al.* (2001) found that a strong correlation exists between urease production (an unusual phenotype for *V. parahaemolyticus*) and the *trh* gene (Iida *et al.*, 2001). In contrast, the *tdh* and *trh* genes were rarely detected in the environmental strains of *V. parahaemolyticus* (Shirai *et al.*, 1990; Kishishita *et al.*, 1992). However, European Commission stated that the practice of judging seafoods exclusively based on total *V. parahaemolyticus* counts without consideration of the virulent factors such as *tdh* and *trh* is not appropriate (Anon, 2001).

An additional genetic target, a thermolabile hemolysis gene (*tilh*) is also present in *V. parahaemolyticus* (Bej *et al.*, 1999) although it is not associated with pathogenicity. However, it has been observed in all of the *V. parahaemolyticus* strains identified, so far (Taniguchi *et al.*, 1986; Bej *et al.*, 1999). Therefore, the species-specific genetic markers viz., *toxR* and *tilh* genes may be adopted for identification of *V. parahaemolyticus*. Besides, a few more molecular characteristics are discussed under the subheading, emergence of O3:K6 serotype.

**Epidemiology (time-line and outbreaks)**

*V. parahaemolyticus* was first identified as a cause of food borne illness in Osaka, Japan in 1950, following the illness of 272 individuals and 20 deaths associated with consumption of semi dried juvenile sardines (Fujino *et al.*, 1970) and has since then been encountered in various parts of the world, including Thailand, Philippines, Australia (Battey *et al.*, 1970), Malaysia and Calcutta (Chatterjee *et al.*, 1970) drawing international attention.

Thereafter, large numbers of *V. parahaemolyticus* strains were detected from the patients with diarrheaa in Calcutta (Sakazaki *et al.*, 1971). In late 1974 and early 1975, 27 passengers of two Caribbean cruise ships got affected consuming sea-foods contaminated with *V. parahaemolyticus* (Lawrence, 1979). The largest reported outbreak in North America in 1997 involving 209 persons was due to eating raw oysters harvested from California, Oregon and Washington in the United States and from British Columbia in Canada where one person died. In the U.S.A, the number of reported outbreaks after 1997 has increased steadily. The total number of food borne outbreaks due to *V. parahaemolyticus* in Tokyo between 1989 and 2000 were 710 (Otaba *et al.*, 2001). The organism was found to be a common cause of foodborne illnesses in many Asian countries, including China (31.1% outbreaks reported between 1991 and 2001), Japan (20–30% of food borne infection cases from 1981 to 1993) and Taiwan (1,495 cases reported between 1981 and 2003, representing 69% of all bacterial food borne outbreaks in this period) (Mauramatsu, 1999; Liu *et al.*, 2004; Nair *et al.*, 2007). In 1999, Hong Kong Department of Health reported a total of 90 confirmed outbreaks of *V. parahaemolyticus* food-poisoning affecting 519 persons and also responsible for 36% of all outbreaks in 2000 (Mok, 2001).

Sporadic outbreaks have also been reported in countries such as Spain (outbreaks reported in 1989, 1999 and 2004) (Lozano-Leon *et al.*, 2003) and France (a serious outbreak reported in 1997) (Robert-Pillot *et al.*, 2004). In a retrospective study on *Vibrio* spp. isolated from patients with diarrhea during 1994 to 2001 in Thailand, prevalence of *V. parahaemolyticus* was reported in 35.5% cases (Srifuengfung *et al.*, 2004). About 300 human cases of infection with *V. parahaemolyticus* caused by consumption of contaminated seafood were reported in Antofagasta, Chile in 1998 (Cordova *et al.*, 2002). Kolkata, an inland metropolitan city on the bank of river Hooghly, is an endemic area for diarrhoeal diseases and *V. parahaemolyticus* was identified as the aetiological agent in 3.5–23.9% of acute human diarrhea cases (Pal *et al.*, 1984). But in most cases, the isolation of *V. parahaemolyticus* from market samples of freshwater fishes was attributed to cross-contamination due to mishandling at fishmongers’ stalls (Sarkar *et al.*, 1985). Since then, the incidence of *V. parahaemolyticus* associated infections has increased with emergence of highly virulent pandemic O3:K6 clone (Okuda *et al.*, 1997; Wong *et al.*, 2000).

**Clinical implications**

Although majority of the strains of varying serotypes of *V. parahaemolyticus* are non-pathogenic but those that are pathogenic causes three major syndromes of clinical illness, i.e., gastroenteritis, wound infections and septicemia. The most common syndrome is gastroenteritis; the symptoms include diarrhea with abdominal cramps, nausea, vomiting, headache, and low-grade fever (Honda and Iida, 1993). Sometimes the diarrhea is bloody, with stools described as meat washed” since the stool is reddish watery stool (Qadri *et al.*, 2003) but unlike that seen in dysentery caused by *Shigella* species or in amebiasis. The mean incubation
period for the infection is 15 h (range, 4 to 96 h). The illness is self-limiting and of moderate severity and lasts an average of 3 days in immunocompetent patients (Qadri et al., 2003). Less commonly, this bacterial species can cause infections in the skin when an open wound is exposed to warm seawater (>15°C). Seven cases of skin infections caused by *V. parahaemolyticus* have been reported in Denmark, which was linked to bathing in the Baltic Sea (Andersen, 2006). Severe wound infections and sepsicaemia have also been reported mainly in immuno-suppressed children and aged people. Individuals who are immune-compromised or burdened with pre-existing health conditions are at high risk for severe complications that can result in death (Morris and Black, 1985).

**Emergence of O3:K6 serotype and its serovariants— their pandemic clonality**

Serotyping based on ‘O’ and ‘K’ antigens can differentiate isolates of *V. parahaemolyticus* and accordingly 13 ‘O’ groups and 71 ‘K’ types are identified by using the commercial antisera (Iguchi et al., 1995).

In the Western coasts of Mexico and the United States, *V. parahaemolyticus* O4:K12 was the dominant serotype in enteric infections (Abbott et al., 1998). Earlier investigations in Calcutta revealed the dominance of serotype O1:K56 among diarrheal cases including carriers (Chatterjee and Sen, 1974; Pal et al., 1984). However, on surveillance study in Calcutta, it was observed that an increase in hospital admissions of patients with *V. parahaemolyticus* gastroenteritis in February 1996. Detailed analysis of the isolates revealed that a unique serotype, O3:K6, which was not previously identified during surveillance in Calcutta, accounted for 50 to 80% of infections from February to August 1996 (Okuda et al., 1997 and Bag et al., 1999). Interestingly, the O3:K6 isolates had identical genotypes (*tdh* positive, *trh* and urease negative) and almost identical arbitrarily primed PCR (AP-PCR) profiles (Matsumoto et al., 2000) and revealed similar antibiotic sensitivity patterns (Okuda et al., 1997).

Isolates of same serovar O3:K6 with similar genotypic traits and AP-PCR profile were also detected in 8 different countries including the United States (Matsumoto et al., 2000). Thus, the Calcutta O3:K6 strains and those isolated from different countries were considered to belong to a single clone (Bag et al., 1999; Chowdhury et al., 2000b and Matsumoto et al., 2000). In Japan, incidence of O3:K6 outbreaks increased from 3% (1988 to 1995) to 75% (1996 to 2001) [Yamasaki et al., 2003]. This alarming rise of O3:K6 serotype that previously associated with only sporadic cases of gastroenteritis was monitored and studied closely in different countries. The O3:K6 clustering of cases differs from the previously reported clustering by showing higher virulence (i.e., rapidly hospitalizations of the patients) and to become the dominant serotype, surpassing other serotypes of *V. parahaemolyticus* in a given area.

Different molecular techniques including AP-PCR, ribotyping and pulsed-field gel electrophoresis revealed that O3:K6 isolates from widely different geographic areas were genetically similar to each other and distinct from O3:K6 isolates obtained before 1995 and from non-O3:K6 serotypes (Nair et al., 2007). The O3:K6 isolates appearing after 1995 carried the *tdh* but not the *trh* gene, did not produce urease (termed new O3:K6), and were defined by possession of a unique sequence (toxRS operon), which encodes transmembrane proteins in the regulation of virulence-associated genes conserved in the genus *Vibrio* (Matsumoto et al., 2000). In Japan, between 1983 and 1988, four *tdh*-negative O3:K6 isolates obtained and revealed similarity (>75%) by AP-PCR and grouped with the unique O3:K6 cluster; moreover, these isolates showed toxRS sequences identical to that of an O3:K6 clone. The findings suggested to infer that the O3:K6 isolates might have originated from these non-pathogenic strains after acquisition of the *tdh* gene (Okura et al., 2003). The corollary findings also suggested to draw the hypothesis that the progenitors of the O3:K6 isolate might have originated in the environs of Japan.

The toxR gene was first discovered in *Vibrio cholerae*, and this gene, when assisted by the toxS gene located immediately downstream, has been shown to be involved in the regulation of many virulence-associated genes in this organism (DiRita, 1992). The toxR gene was also detected at least in *V. parahaemolyticus*, *Vibrio fischeri*, *Vibrio vulnificus* and *Vibrio hollisae* and its analyses supported to presume that the toxR is a global regulatory gene conserved in the members of the genus *Vibrio*. The toxR sequence identity between *V. parahaemolyticus* and other *Vibrio* species is considerably lower than the sequence identities for icon genes viz. 16S rRNA, gyrB. It suggested the yardstick of toxR gene for identification of *V. parahaemolyticus* at the species level. Like *V. cholerae*, the toxRS operon of *V. parahaemolyticus* encodes the transmembrane proteins involved in the regulation of virulence-associated genes. The old O3:K6 strains (isolated before 1995) and new O3:K6 (isolated since 1995) were analysed for variation in nucleotide sequence of the toxRS region. The difference in the sequence between the new and the old O3:K6 strains ranged from 11 to 14-bp within the 1,364-bp region covering 95.4% of the toxRS coding regions, and the sequences of these two groups (old O3:K6 and new O3:K6 clone) differed invariably at 7 base positions (Matsumoto et al., 2000). Moreover, conservation of the 7 bases in the new O3:K6 clone was
further confirmed by determining the stretches of the sequences including these 7 bases (Matsumoto et al., 2000). Restriction endonuclease analyses for a limited number of O3:K6 strains isolated from 1995 indicated that 2 of 7 unique bases in the toxRS sequence could be incorporated in designing the sense and anti-sense primers to accomplish a PCR to amplify the toxRS region of the O3:K6 new clone. Based on the available data a PCR method, referred to as the group specific PCR (GS-PCR) was designed to distinguish the new O3:K6 strains from the old O3:K6 strains generating the 651-bp amplicon. It is accepted that the development of GS-PCR was a milestone in simplifying the identification of the O3:K6 isolate and also for detecting other serotypes that share identical molecular traits (Matsumoto et al., 2000).

GS-PCR of non O3:K6 strains presented interesting data with other serovars O4:K68, O1:K25 and O1:K untypable (KUT) producing the 651-bp amplicon with the GS-PCR specific primers. Strains belonging to these serovars were not only genotypically similar to the new O3:K6 strains but they also exhibited AP-PCR profiles indistinguishable from that of the new clone. The results indicate that the GS-PCR-positive strains belonging to the O4:K68 and O1:KUT serovars are genetically very close to the new O3:K6 clone (Matsumoto et al., 2000). The O4:K68 serovar has never reported before in the list of known O:K serovars. The strains of this serovar were first isolated in 1997 from International travellers (Okuda et al., 1997) and were subsequently detected in India (March 1998) (Bag et al., 1999), Bangladesh and Japan (Matsumoto et al., 2000).

Although strains of serovar O1:KUT have been detected since 1980, GS-PCR-positive O1:KUT strains first appeared in India in 1997 and subsequently detected in Bangladesh and from an international traveller. Besides, another serotype, O6:K18, which shared high molecular identity with O3:K6 isolate was detected in Taiwan (Wong et al., 2005). Therefore, it may be assumed that the other serotypes (O4:K68, O1:K25, and O1:KUT, O6:K18) that had identical genotypes and molecular profiles to those of O3:K6 isolates emerged from O3:K6 serotype and these were collectively referred to as “serovariants” of O3:K6 isolates (Matsumoto et al., 2000). Therefore, these toxRS (GS-PCR)-positive serotypes appeared to have diverged from the O3:K6 isolates by alteration of the O:K antigens and were postulated to be clonal derivatives of the O3:K6 serotype and followed a spreading pattern similar to the new O3:K6 clone (Chowdhury et al., 2000a; Matsumoto et al., 2000). Hence, the infection caused at least by these serovars, O3:K6, O4:K68 O1:KUT and O6:K18, may be categorized as an emerging infectious disease, considering the extent of their geographical spread thus comprising the first described pandemic in the history of this organism (Matsumoto et al., 2000). In a survey of clinical diarrhea in Khanh Hoa Province in Vietnam, eleven (CDC, 1999) O:K serotypes were detected among the recovered isolates, and all were found to be closely related to O3:K6 (Chowdhury et al., 2004). Till date, a total of 13 serotypes (O1:KUT; O4:K68; O1:K25; O5:K25; O1:K33; O2:K3; OUT;KUT; O3:KUT; O3:K5; O4;K4; O4;K10; O5;KUT and O5;K17) have been identified in India that are found similar to O3:K6 serotype by a variety of molecular typing techniques (Nair et al., 2007). Han et al. (2008) studied the genome plasticity of V. parahaemolyticus and concluded that the depiction of an evolutionary history of the pandemic group (clone), strains of new-O3:K6 and its serovariants (post-1996 O4:K68, O1:K25, O1:KUT and O6:K18) constituted the pandemic group and New-O3:K6 (trh+, tdh+ and GS-PCR+) was emerged from the old-O3:K6 clone (trh+, tdh+ and GS-PCR+) by the stepwise acquisition of genomic islands.

Chronicle for global spread of O3:K6

In 1998 in Japan, isolation of this pathogen in foodborne infections doubled compared to that in 1997 and exceeded the number of infections by Salmonella - the major cause of food-borne infections (WHO, 1999). An increase in food-borne disease outbreaks was recorded in Taiwan in 1996 with isolation of V. parahaemolyticus O3:K6 in high rate (Chiou et al., 2000, Wong et al., 2000). During 1995, in Taiwan, the O3:K6 serotype accounted for only 0.6% of V. parahaemolyticus infections, and this level abruptly increased to 50.1% in 1996 and reached a peak of 83.8% in 1997 (Chiou et al., 2000). Since 1996, the O3:K6 serotype, was isolated from Bangladesh (Bhuiyan et al., 2002; Chiou et al., 2000; Matsumoto et al., 2000). During May and June 1998, a total of 416 persons in 13 states suffered with gastroenteritis after eating oysters harvested from Galveston Bay, Tex., and their 28 available stool samples yielded V. parahaemolyticus O3:K6 isolates, which closely resembled the Asian O3:K6 isolates by PFGE (Daniels et al., 2000). Similarly, during July to September 1998, an outbreak of V. parahaemolyticus O3:K6 infections associated with the consumption of oysters and clams harvested from Long Island Sound occurred among residents of Connecticut, New Jersey, and New York (CDC, 1998; 1999). Before this, outbreaks with V. parahaemolyticus serotype O3:K6 in the United States was not reported (Daniels et al., 2000). Subsequently, O3:K6 isolates obtained in 1997 and 1998 from clinical sources in Taiwan, Laos, Japan, Thailand, Korea, and the United States were found to share nearly identical AP-PCR profiles (Matsumoto et al., 2000). From November 1997 to March 1998, O3:K6 serotype reported in outbreaks in the northern city of Antofagasta, Chile and other outbreaks occurred during the summer months of 2004 and 2005, mainly in Puerto Montt, a region with usually
cold waters (Cordova et al., 2002, Fuenzalida et al., 2006; Gonlez-Escalona et al., 2005).

V. parahaemolyticus O3:K6 serotype recovered in 1996 from several places in Peru and subsequently, found prevalent of this serotype and its other pandemic serovariants (Gill et al., 2007). Interestingly, in 1996 the O3:K6 serotype was also identified in diarrhoeal outbreak in Kolkata, India (Nair et al., 2007). The majority of the V. parahaemolyticus strains isolated during an outbreak of acute enteric disease in Vladivostok, Russia, in 1997 belonged to serotype O3:K6 (Smolikova et al., 2001). More recently, the O3:K6 serotype was isolated from clinical diarrhea cases in Mozambique, indicating its spread into the African continent (Ansaruzzaman et al., 2005). V. parahaemolyticus strains similar to the pandemic clone have been isolated from the coasts of Spain and France (Martinez-Urtaza et al., 2004; Quilici et al., 2005). Such occurrence clearly supports the global dissemination of a specific clone of V. parahaemolyticus.

In Kolkata, V. parahaemolyticus isolated from travellers, returning to Japan in 1996, were compared with the group of isolates obtained till 1993. It was observed that Kolkata O3:K6 isolates were identical to the isolates obtained from travellers from 1995 onwards but differed from O3:K6 isolates isolated prior to 1993. Further, it was also revealed that like Kolkata similar O3:K6 were already prevalent in Indonesia, Thailand, and Singapore, since travellers returning from these countries were infected (Okuda et al., 1997). Based on chronological data, Matsumoto et al. (2000) concluded that the O3:K6 serotype (new) emerged only recently, who showed that O3:K6 isolates from clinical sources in Taiwan, Laos, Japan, Thailand, Korea, and the United States in 1997 and 1998 were identical to the Kolkata O3:K6 isolates. With this evidence, they concluded that a widespread occurrence of one clonal type of V. parahaemolyticus was unprecedented and the unique O3:K6 isolate and its serovariants were causing pandemic.

Conclusion

Increasing occurrence of V. parahaemolyticus O3:K6 and its serovariants globally, since 1995, gained attention in public health research. However, of late, hospitalisation of such patients and their sufferings are recorded to be lower than earlier. This trend warrants to pay more attention for intensive study on few aspects of this organism viz. about perpetuation of this organism in environment, mode of acquiring of virulence and evolving of O3:K6 serotypes and its variants, tangible decrease in degree for expression of clinical symptoms etc. in order to tackle public health problems caused by this organism.

Acknowledgements

The authors are thankful to the Director, IVRI, Izatnagar, Dr. GB Nair, Executive Director, Translational Health Science & Technology (DBT), Gurgaon, Haryana, India and Dr. T Ramamurthy, Deputy Director (SG), NICED (ICMR), Kolkata for their benevolent support and encouragement in preparing the manuscript.

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